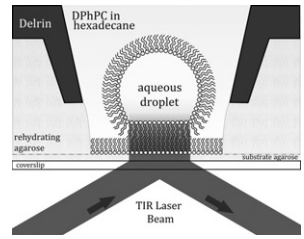
1717-Pos Board B627
In Vitro Tracking of the Gold Nanoparticles
Cemil Boyoglu, Seyhan B. Barnum, Shivani Soni, Vida A. Dennis, Shree R. Singh.
Gold nanoparticles are nanomaterials with desirable properties, such as ease of preparation and bioconjugation, efficient resistance to surface oxidation and unique optical properties. These properties make them ideal probes for biological and cell imaging applications. In this study, we conducted a series of studies using different sizes of gold nanoparticles, including 3.5nm, 10 nm, 26nm and 50 nm, to determine if the size of the nanoparticle affects cell entry. We also tracked the movement of the gold nanoparticles with different microscopic techniques, such as Confocal Laser Microscope, Atomic Force Microscobe, Scanning Electron Microscobe and Transmission Electron Microscobe. We used HEp-2 cell lines and stained the nucleus, mitochondria, golgi apparatus, and plasma membrane using different stains to track the gold nanoparticles movement in the cell. We found that gold nanoparticles exhibited different behaviors depending on their sizes, and are located in different organelles during different times (1h, 2h, 4h, 8h, 12h, 24h, 48h, and 72h). Our confocal study results showed that as the size of the gold nanoparticles increases, the time of entering the cells was delayed. We also observed that nanaparticles occupied different organelles as time incubation periods increased. For example, 3.5nm gold nanoparticles accumulated on the plasma membrane after 1 h of incubation, then moved to the mitochondria after 4h of incubation and then to the nucleus after 24 h of incubation. In contrast, 10nm gold nanoparticles were not near the plasma membrane even after 2h incubation and were primarily in the mitochondria after 24 h of incubation.

1718-Pos Board B628
Lens Design for Small Diameter in Vivo Endoscopic Multiphoton Microscopy
Christopher M. Brown, Chris Xu, Watt W. Webb.
Multiphoton microscopy has demonstrated the capacity to image tissue anatomy and morphology with high resolution optical depth sectioning and the potential for diagnosis of tissue health. We are building devices to transition multiphoton imaging and diagnostic techniques from the laboratory to the clinic and hospital using in vivo multiphoton endoscopy. In this instrument, lens design for a miniature (< 5 mm outer diameter) endoscopic multiphoton microscopy system presents a significant challenge. Lens system design goals include: wide field of view and moderate resolution image acquisition, high epifluorescent collection efficiency from scattering media, design for use with scanned optical fiber imaging systems, small device size and low cost manufacture. We present results for a three-successive element lens design that meets these criteria.

1719-Pos Board B629
Cellular Binding of Nanoparticles in the Presence of Serum Proteins
Gerard W. Doorley, Christine K. Payne.
Nanoparticles have important biomedical applications ranging from the treatment of human disease with gene therapy to understanding basic cellular function. For cellular uptake of nanoparticles to occur, the particle must first bind to the cell surface. We sought to understand how nanoparticles interact with the cell surface in the presence of serum proteins. Cell culture medium supplemented with serum is a highly complex mixture of amino acids, vitamins, inorganic salts, glucose and proteins. Characterization of cationic, fluorescent nanoparticles in the presence of cell culture medium demonstrated that the particles rapidly bind a mixture of proteins present in the serum resulting in an anionic nanoparticle that essentially presents a surface of serum proteins to the cell surface. Probing the interaction of this serum protein-nanoparticle complex with the cell surface was achieved using fluorescently labeled proteins and monitoring the colocalization of nanoparticle and protein signal with two-color fluorescence microscopy over time. Our results indicated that both nanoparticle and protein were highly colocalized at early times and this was found to decrease with increased incubation time. Displacement of the serum proteins from the nanoparticles was found to be protein dependent. This study has important implications for the rational design of nanoparticles and ligands for delivery in a cellular environment as it demonstrates the significance of serum protein-nanoparticle interactions. Such interactions can ultimately influence the cellular binding of the nanoparticle to the cell surface.[1]
[1] G. W. Doorley and C. K. Payne, '' Cellular Binding of Nanoparticles in the Presence of Serum Proteins'' Chem. Commun., 2010, in press

1720-Pos Board B630
TIRF Imaging of Lipid Bilayer Arrays
Oliver K. Castell, James M. Berridge, Mark I. Wallace.
A TIRF imaging platform for the simultaneous interrogation of multiple lipid bilayers in an arrayed format has been developed. Here, a proof of principle demonstration is made with the quantification of Calcium flux through the trans membrane pore, alpha-hemolysin. The effect of pore blocking by cyclodextrins is also demonstrated.
Utilising a droplet on hydrogel method of lipid bilayer formation, an array of multiple lipid bilayers are illuminated simultaneously by the evanescent field of laser light undergoing total internal reflection at the surface of the glass hydrogel support layer.
The droplet based method of bilayer production is ideally suited to an array platform, with the segregated contents of each droplet affording the opportunity to incorporate various membrane proteins, or simultaneously screen a variety of molecular candidates for their interaction with incorporated membrane components. The system offers the opportunity of asymmetric bilayer generation and the option of rapidly screening environmental dependencies for protein function. The scalable and automatable nature of the approach, together with the ability to tailor droplet contents, creates many exciting opportunities for characterisation of membrane protein behaviour and ligand interactions, the focus of future studies with the platform.



1721-Pos Board B631
Caveolae Play an Active Role in Mediating Ca^{2+} Activity in Adult Cardiac Myocytes Through Stabilization of $Galpha_q$
Urszula P. Golebiewska, Yuanjian Guo, Suzanne Scarlata.
Cardiomyocytes have complex Ca^{2+} behavior that can range from waves propagating through the cell to small localized spikes. Intracellular Ca^{2+} activity can be initiated by second messengers generated by the phospholipase C-beta- $G\alpha_q$ pathway. Previous work suggested that the caveolae structural proteins, caveolin-1 or caveolin-3 (Cav3) may specifically interact with activated $G\alpha_q$ and effect Ca^{2+} signals. Here, we show that stimulation of cardiomyocytes does not affect the co-localization of Cav3 and $G\alpha_q$ but reduces the co-localization of Cav3 and Gβ3. This reduction is accompanied by increased Gβγ mobility suggesting that Gβγ subunits are released from caveolae upon stimulation. Studies using a fluorescence sensor that reports on activated $G\alpha_q$ and competing peptide suggest that Cav3 stabilizes the activated state of $G\alpha_q$. To determine the impact of Cav3- $G\alpha_q$ interactions on Ca^{2+} activity, we monitored myoctyes using a fluorescent Ca^{2+} sensor. We find that adult cardiomyocytes have slowly oscillating Ca^{2+}waves that are not seen in neonatal cells which do not contain Cav3. Microinjection of a peptide that disrupts Cav3- $G\alpha_q$ association extinguishes these waves but a control peptide has no effect. Also, these waves are not seen when the cells are treated with a PLC inhibitor but not ryanodine implying that Cav3- $G\alpha_q$ are responsible for this Ca^{2+} activity. Taken together, these studies show that caveolae play an active role in Ca^{2+} signaling through their stabilization of activated $G\alpha_q$ and not simply through localizing proteins.

1722-Pos Board B632
Quantification of in Vivo Left Ventricular Torsion and Principal Strains in Mouse Models of Hypertrophic and Dilated Cardiomyopathy
Candida L. Desjardins, Yong Chen, Arthur Coulton, Salman Azam, Brian Hoit, Xin Yu, Julian Stelzer.
The deformations and twisting of the left ventricular (LV) wall, quantified by strain and torsion, provide insight into its regional and global contractile function. In this study, we investigated the influence of proteins entailing myocyte contractility and structural stability on ventricular biomechanics using mouse models with alterations in contractile proteins. The pattern and timing of LV strain and torsion in 2~3 month mice that lack cardiac myosin binding protein-C (cMyBP-C-/-, n=6, cMyBP-C+/-, n=6), a thick filament-associated sarcomeric protein, and muscle LIM protein (MLP-/-, n=6), a cytoskeleton protein that is thought to be involved in transmission of mechanical stress, were evaluated against wild-type mice (n=6) in vivo using magnetic resonance imaging. Both cMyBP-C-/- and MLP-/- mice exhibited a severe depression in systolic function as indicated by decreased LV ejection fractions compared to wild-type mice. A significant reduction in LV torsion and principal strains E1 and E2, associated with radial wall thickening and circumferential shortening, respectively, were observed in cMyBP-C -/- and MLP-/- mice. Interestingly,